REMARKS

Summary of Amendments

Claims 1, 6, 12-20, 23, 24, 26, 27, and 31 are pending in this application. All claims have been rejected in the Office Action of October 23, 2000. In this Reply, Applicants have added Claims 32-34 and have amended Claims 1, 6, 13, 14, 16, 17, 18, 26, 27 and 31.

The amendment to claim 1 reciting "with the predetermined target" is supported in step (a)(i) of the same claim. This recitation is further supported in the legend for Fig. 2 (page 6; employing the phrase "known target protein"), p. 10 lines 10-12 (exemplified by showing that cyclooxygenase is the target for a modified aspirin), and further specified in the numerous examples provided in Table 2 (page 22). The amendment to claim 13 is intended more particularly to point out and more distinctly claim the present invention, and establishes the antecedent basis for the recitation of claim 14. The latter amendments inserting the word "random" are supported at least at page 4, line 16. The amendment to claim 31 is found at least on page 4, lines 11-12. Support for claims 32-34 is found on pages 9-13. No new matter has been added by these amendments.

In the Combined Declaration and Power of Attorney

The Examiner noted in Item 10 on page 4 of the Office Action that the address of Inventor Vimal D. Mehta had been altered without initials. A Supplemental Combined Declaration and Power of Attorney is filed herewith as required under 37 C.F.R. §§ 1.52(c) and 1.121(a), in accordance with MPEP § 602.02, to correct this oversight. Taken together, the Combined Declaration and Power of Attorney as originally filed and the Supplemental Combined Declaration and Power of Attorney filed herewith provide all required data under 37 C.F.R. § 1.63. Applicants believe no further documentation is needed.

In the Specification

In response to Item 12 of the Office Action, the amendment on page 10 is made to correct a grammatical error and to further clarify that "Sigma" is not a trademark as stated by the Examiner, but rather is the name of a biochemical reagent supplier.

Rejections Under 35 USC §112, Second Paragraph

Claims 1, 6, 13, 14, 16, 17, 18, 26, 27, and 31 have been rejected under 35 USC § 112 as being indefinite for failing to particularly point out and distinctly claim the subject matter of the invention. Without acceding to the propriety of the Examiner's rejections, Applicants have amended the claims in order to facilitate prosecution. Claims 1, 13, 14, 16, 17, 26, and 31 have been amended to provide proper antecedent bases and to use the same terms throughout the claims. Further support for amendments to Claim 1 appears in the specification, *e.g.*, at least at page 4, lines 8 to 19. Claim 18 has been made definite by removing the parenthetical phrase, as supported in the specification, *e.g.*, at least at page 11, lines 15 to 21. New claims 32-34 are supported by original claim 18 and in the specification, *e.g.*, at least at page10, lines 13 to 15, and page 20, lines 5 to 15 for claim 32; at least at page 12, lines 3 to 5, for claim 33; and at least at sections 1-4 on page 9, line 19, to the bottom of page 14. Claim 27 has been amended to correct typographical and grammatical errors. Claim 31 has been amended to more distinctly claim the kit. Applicants believe the rejections under 35 USC § 112, second paragraph, are now moot as applied to the claims as amended, and request that the rejections be withdrawn.

Summary of Applicants' Invention

The instant invention relates to a method for identifying a cellular component to which a small molecule is capable of binding. An important feature of the presently claimed method is that a potentially three-component yeast hybrid system (in which the three components are the first hybrid protein, the second hybrid protein and the hybrid ligand) is reduced to a two component system by virtue of the formation of a covalent bond between the target protein (a component of the first hybrid protein) and ligand A, which is a component of the hybrid ligand. (See page 9, lines 1-10). In this system, the two components are (a) the first hybrid protein covalently linked to the hybrid ligand, and (b) the second hybrid protein.

Rejections Under 35 USC § 102 (b) and (e)

The Examiner has rejected claims 1, 6, 12, 17, 19, 20, and 23-24 under 35 USC § 102(b) as being anticipated by Licitra *et al* ("Licitra") and claims 1, 6, 12-17, 19, 20, 23, 24, 26, 27, and 31 under 35 USC § 102(e) as being anticipated by U.S. Patent No. 5,928,868 to Liu *et al* ("Liu"). Applicants traverse the rejections for the reasons described below.

Licitra 102(b) Rejection

The Examiner states that Applicants' claims are directed to a three-hybrid system and that Licitra discloses each element of such a three-hybrid system. Applicants respectfully disagree with this position.

As noted in the Office Action, Applicants cited Licitra on Form PTO-1449 filed 12/9/99. Licitra describes a three-component yeast hybrid system. This is apparent by inspection of, for example, Fig. 2 (page 12819, col. 2), as well as numerous references to a three-hybrid system (page 12817, the title of the article; page 12817, col. 1, Abstract; p. 12817, col. 2, 1st paragraph; p. 12818, col. 2, bold font heading of second paragraph; and so forth). Applicants' claims recite that ligand A forms an irreversible (covalent) bond with a predetermined target for which the ligand A has specificity. See claim 1 step (a). The claims also recite that the hybrid ligand molecule binds covalently the first hybrid protein through ligand A. See claim 1(c). Such an irreversible bond between ligand A and its predetermined target results in changing the three hybrid system to a two-hybrid type of system, because the hybrid ligand becomes irreversibly bound to the target for ligand A. Nowhere in Licitra is it disclosed that ligand A has a specificity for a predetermined target and forms an irreversible (covalent) bond, as presently claimed by Applicants. Licitra has not provided the required reduction of the three-hybrid system to a two-hybrid system. Since Licitra does not teach the recited claim element of irreversible (covalent) binding between a ligand A and its predetermined target, Licitra does not anticipate the claimed invention. Applicants thus believe that the 102(b) rejection over Licitra has been overcome and request that this rejection be withdrawn.

Liu 102(e) Rejection

The Examiner states that Applicants' claims are directed to a three-hybrid system and that Liu discloses each element of such a three-hybrid system. Applicants respectfully disagree with this position.

Liu describes a three-component yeast hybrid system. This is apparent by inspection of, for example, Fig. 2 (described as "a diagrammatic representation of the components of the three hybrid assay" (col. 4, lines 13-14)), Fig. 3 (described as "a diagrammatic representation of a generalized sequence of events during a three hybrid screen" (col. 4, lines 19-20), as well as

numerous references to a three-hybrid system (title of the patent; col. 3, line 16; col. 3, line 26; col. 3, line 55; col. 3, line 61; and so forth).

Applicants' claims recite that ligand A forms an irreversible (covalent) bond with a predetermined target for which the ligand A has specificity. See claim 1 step (a). The claims also recite that the hybrid ligand molecule binds covalently the first hybrid protein through ligand A. See claim 1 step (c). Such an irreversible bond between ligand A and its predetermined target results in changing the three hybrid system to a two-hybrid type of system, because the hybrid ligand becomes irreversibly bound to the target for ligand A.

Applicants believe, therefore, that the claims are not anticipated by Liu and request that the 102(e) rejections be withdrawn.

Rejections Under 35 USC § 103 (a)

The Examiner has rejected claims 1, 6, 12, 17, 19, 20, 23-24, and 31 under 35 USC § 103 (a) as being rendered obvious (1) by the disclosure of Licitra, and (2) by the disclosure of Crabtree *et al.* (WO 94/18317; "Crabtree") in view of Wickens *et al.* (US Patent No. 5,610,015; "Wickens").

1) Rejection over Licitra

Although all of claims 1, 6, 12-17, 19-20, 23-24, 26-27 and 31 are cited as being obvious over Licitra, the *prima facie* case set forth in the Office Action relates only to claim 31, drawn to a kit. As noted in the Office Action, Applicants cited Licitra on Form PTO-1449 filed 12/9/99. Claim 31 as amended recites the feature that ligand A has a specificity for a predetermined target and forms an irreversible (covalent) bond. Licitra does not disclose, motivate or suggest the providing of a preactivated ligand A and reagents for forming a hybrid molecule with at least one type of ligand B, wherein ligand A has a specificity for a predetermined target and forms an irreversible (covalent) bond. Accordingly, Licitra does not render claim 31 obvious. Similarly, Licitra does not teach, suggest, or motivate a skilled artisan to provide a method for identifying a small molecule that binds to cellular component in which a hybrid ligand consists essentially of two ligands, identified as ligand A and ligand B that are linked together, wherein ligand A has a specificity for a predetermined target and forms an irreversible (covalent) bond; and ligand B is the small molecule that is to be identified as binding to the cellular component. Accordingly, Licitra fails to render claims 1, 6, 12-17, 19-20, 23-24, and 26-27 obvious as well. Applicants therefore conclude that all of claims 1, 6, 12-17, 19-20, 23-24, 26-27 and 31 as amended are nonobvious over Licitra. Withdrawal of this rejection is requested.

2) Rejection Over Crabtree in View of Wickens

As noted in the Office Action, Applicants cited Crabtree and Wickens on Form PTO-1449 filed 12/9/99. Crabtree relates to a procedure for the regulated (inducible) dimerization or oligomerization of intracellular proteins. The proteins are induced to associate by treating the cells containing them with synthetic ligands (see Abstract). The method employs ligands that are capable of binding to two (or more) receptor domains, with specified values of Kd below certain minima (see page 3, lines 21-28). By specifying a Kd value, the inventors are understood to be discussing a reversible binding system, i.e., a ligand and its receptor. They are not describing a system in which a ligand binds irreversibly to a target; formation of such a covalent bond would not be described in terms of a Kd value. The depiction of a reversible three-component system is provided in Fig. 14, which also makes clear, by the use of bi-directional arrows, that the binding processes are reversible.

Wickens discloses a method for detecting an interaction between an RNA-binding protein and a test RNA molecule. This system also is a three-component system in which, instead of one component being a hybrid ligand, it is an RNA molecule. The binding intermediates are depicted in Fig. 1, panels B and C. Applicants respectfully point out that the interactions described in this method are reversible interactions. This is borne out at least by use of the phrase "...determine which test RNA sequence bound the RNA-binding protein with the highest affinity." (col. 4, lines 43-45). The term "affinity" as commonly applied to chemical and biochemical interactions and processes relates to the notion of a reversible binding interaction characterized by an association constant Ka or its inverse, a dissociation constant Kd. This usage of Kd relates to the same usage of a dissociation constant Kd as employed in Crabtree. In summary, both Crabtree and Wickens disclose systems employed in the detection of reversible interactions. Neither Crabtree nor Wickens teach, suggest or motivate a skilled artisan to provide a method for identifying a small molecule that binds to a cellular component in which a hybrid ligand consists essentially of two ligands, identified as ligand A and ligand B that are linked together, wherein ligand A has a specificity for a predetermined target and forms an irreversible (covalent) bond with the predetermined target; and ligand B is the small molecule that is to be identified as binding to the cellular component.

There is an additional deficiency in each of Crabtree and Wickens. Crabtree does not teach or motivate developing a method for identifying a cellular component to which a small molecule is capable of binding. Rather, Crabtree discloses DNA constructs encoding a chimeric protein comprising a receptor domain capable of binding to a selected ligand, fused to a heterologous additional protein domain capable of initiating a biological process upon exposure to the ligand. Crabtree also discloses a method for activating the transcription of a target gene in cells by providing cells expressing a protein such as just described which further includes a domain capable of initiating a detectable intracellular signal upon exposure to the ligand. Importantly, Crabtree employs known protein domains and known ligand moieties that bind to the domains. The reference does not relate to a method for identifying a cellular component to which a small molecule is capable of binding; i.e., it does not seek to identify a new ligand or a new receptor by a screening procedure.

Wickens discloses the use of first, second and third chimeric genes. The third chimeric gene comprises a first RNA sequence capable of binding to either the first or second RNA-

binding domain in the first or second chimeric gene product, and a second RNA sequence to be tested for interactions with the RNA-binding protein not bound to the first RNA sequence. (see Abstract). Thus Wickens teaches the use of RNA as the ligand whose binding is being tested or characterized. RNA is not a small molecule. Since the residue weight of a single nucleotide in a nucleic acid is about 330 D (daltons), an RNA molecule such as disclosed in Wickens, for example as is depicted in Fig. 1, Panels B and C, must have a molecular weight of more than about 10,000 D, and probably more than about 50,000 D. In contrast, the small molecule ligands A and B of the present invention are defined as having molecular weights of less than 1000 D and greater than 50 D (page 15, lines15-16). The small molecules are shown, by way of nonlimiting examples, by dexamethasone, FK506, cyclosporin, and so on, each with molecular weights less than about 1,000D. These examples clearly are not nucleic acids of any kind.

Applicants find no motivation in Crabtree to combine with Wickens, or vice versa. Furthermore, even if these references were to be so combined, they would not provide the invention of the present claims. A method for activating the transcription of a target gene in cells using a three-component system all of whose components are known (Crabtree) cannot be combined with a method for screening for a protein that interacts with an RNA moiety (Wickens) to provide the instantly claimed method. The proposed combination would not provide a method for identifying a cellular component to which a small molecule is capable of binding, in which method a three-hybrid system is reduced to a two-hybrid system by virtue of the formation of a covalent bond between the target protein that is a component of the first hybrid protein and ligand A, which is a component of the hybrid ligand.

For the reasons presented above, the combination of Crabtree and Wickens fails to render claims 1, 6, 12-20, 23-24, 26-27 and 31 obvious. Accordingly, Applicants request that these rejections be withdrawn.

CONCLUSION

Applicants submit that the Examiner's rejections have been overcome based on the enclosed amendments and remarks. Applicants therefore respectfully request that claims 1-6, 12-20, 23-24, 26-27 and 31-35 be found allowable at this time. Should any questions or issues arise concerning the application, the Examiner is encouraged to contact Applicants' undersigned attorney at the telephone number indicated below.

This Reply is in response to the Office Action mailed October 23, 2000. A two-month Petition for Extension of Time, together with a check in the amount of $195.00 to cover the extension fee, is being filed simultaneous with the filing of this response. With the extension of time, these documents are due on or before March 23, 2001. Please charge any payments due, or credit any overpayments of same, to Deposit Account No. 50-0311, reference 15966-518.

Respectfully submitted,

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VERSION MARKED TO SHOW CHANGES

In the Specification:

At page 10, line 14, please insert after the word "Sigma" the phrase --Chemical Co., St. Louis, MO--, shown as follows:

Modified aspirin (aminoalkyl salicylates) were synthesized as shown in FIG. 3. The Dexamethasone (Sigma Chemical Co., St. Louis, MO) and FK506 (Fujisawa Pharmaceuticals) were linked to aminoalkyl salicylates to form a hybrid molecule. The chemistry utilized to effect the linkage is shown in FIG. 3. The dexamethasone and FK506 hybrid molecule with aminosalicylates were synthesized utilizing synthetic transformations outlined in FIG. 3. The dexamethasone portion of the hybrid molecule was synthesized as dexamethasone free amine starting from commercially available dexamethasone in three synthetic modifications (Licitra, et. al., PNAS 93, 12817, 1996). The FK506 portion of the hybrid molecule was synthesized as the N-hydroxysuccinamide activated ester from the natural product FK506 in a total of four synthetic modifications [(](Licitra, et al., PNAS 93, 12817, 1996). The dexamethasone amine (and FK506 activated ester) were coupled to aminosalicyclates as shown in FIG. 3.

Please amend claims 1, 6, 13, 14, 16, 17, 18, 26, 27 and 31, as follows:

1. (Amended) A method for identifying a cellular component to which a small molecule is capable of binding, comprising:

(a) providing a hybrid ligand consisting essentially of two ligands, identified as ligand A and ligand B that are linked together, wherein

(i) ligand A has a specificity for a predetermined target;

(ii) ligand A [and] forms an irreversible (covalent) bond with the predetermined target;

(iii) and ligand B is the small molecule;[:]

(b) introducing the hybrid ligand [molecule] into at least one sample, the sample containing an environment, the environment containing;

(i) a first expression vector, comprising [including] DNA encoding the target for ligand A, linked to a coding sequence for a first transcriptional module for expression as a first hybrid protein;

(ii) a second expression vector comprising [including] a random DNA fragment encoding a polypeptide linked to a second transcriptional module for expression as a second hybrid protein; and

(iii) a third vector comprising [including] a reporter gene wherein the expression of the reporter gene is conditioned on the proximity of the first and second hybrid proteins;

(c) permitting the hybrid ligand [molecule] to bind covalently the first hybrid protein through ligand A and the second hybrid protein through ligand B so as to activate the expression of the reporter gene;

(d) identifying those samples expressing the reporter gene; and

(e) characterizing the second hybrid protein in the samples identified in (d) so as to determine the cellular component to which the small molecule has a binding affinity.

6. (Amended) A method according to claim 1, wherein the environment in step (b) is selected from the [a] group consisting of insect cells, yeast cells, mammalian cell, and their lysates.

13. (Amended) A method according to claim 1, wherein the second expression vector of step (b)(ii) contains a random DNA fragment of a size suited for encoding a gene product wherein said random [the] DNA fragment is from a library of DNA.

14. (Amended) A method according to claim 13, wherein the random DNA fragments in the library are selected from the group consisting of genomic DNA, cDNA and synthetic DNA.

16. (Amended) A method according to claim 14, wherein the [cDNA] library is a cDNA library derived from an immune cell.

17. (Amended) A method according to claim 16, wherein the cDNA is derived from an immune cell capable of producing an immune response to a [the] small molecule contaminant.

18. (Amended) A method according to claim 1, wherein the ligand A or B of step (a) is a mechanism-based irreversible enzyme inactivator [(*e.g.*, aspirin-cyclooxygenase)].

26. (Amended) A method according to claim 1, wherein the [cell] cellular component is a protein.

27. (Amended) A method according to claim 24, wherein the steps (b) - (e) of the method are repeated using an expression vector encoding the second hybrid protein of step (e) and a hybrid ligand [molecule] containing ligand A and ligand B[.] in the presence of a preparation of random small molecules that bind competitively to [for competitive binding with] the hybrid ligand [molecule] and identifying the small molecule capable of competitively binding the target molecule for searching for new target molecules in an iterative process.

31. (Amended) A kit for detecting interactions between pharmacologically relevant small molecules and proteins comprising;

(a) a preactivated ligand A and reagents for forming a hybrid ligand [molecule] with at least one type of ligand B, wherein ligand A has a specificity for a predetermined target and forms an irreversible (covalent) bond with the predetermined target;

(b) a first expression vector comprising [including] DNA encoding [the] a target [binding protein] for [Ligand] ligand A linked to a coding sequence for a first transcriptional module for expression as a first hybrid protein;

(c) a second expression vector comprising [including] a random DNA fragment encoding a polypeptide linked to a coding sequence for a second transcriptional module for expression as a second hybrid protein;

(d) a third vector comprising [including] a reporter gene wherein transcription of the reporter gene is conditioned on the proximity of the first and second hybrid [target] proteins;

(e) an environment for transcription and translation of the first and second hybrid proteins and reporter genes; and

(f) a means for detecting the expression of the reporter gene following the formation of a trimeric complex between the hybrid ligand and the first and second hybrid proteins.